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ORIGINAL ARTICLE

Musculoskeletal

Advanced magnetic resonance imaging of cartilage components in haemophilic joints reveals that cartilage hemosiderin correlates with joint deterioration

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Abstract

Introduction: Evidence suggests that toxic iron is involved in haemophilic joint destruction.

Aim: To determine whether joint iron deposition is linked to clinical and imaging outcomes in order to optimize management of haemophilic joint disease.

Methods: Adults with haemophilia A or haemophilia B (n = 23, ≥ age 21) of all severities were recruited prospectively to undergo assessment with Hemophilia Joint Health Scores (HJHS), pain scores (visual analogue scale [VAS]) and magnetic resonance imaging (MRI) at 3T using conventional MRI protocols and 4-echo 3D-UTE-Cones sequences for one affected arthropathic joint. MRI was scored blinded by two musculoskeletal radiologists using the International Prophylaxis Study Group (IPSG) MRI scale. Additionally, UTE-T2* values of cartilage were quantified. Correlations between parameters were performed using Spearman rank correlation. Two patients subsequently underwent knee arthroplasty, which permitted linking of histological findings (including Perl's reaction) with MRI results.

Results: MRI scores did not correlate with pain scores or HJHS. Sixteen joints had sufficient cartilage for UTE-T2* analysis. T2* values for cartilage correlated inversely with HJHS (r_s = −0.81, P < 0.001) and MRI scores (r_s = −0.52, P = 0.037). This was unexpected since UTE-T2* values decrease with better joint status in patients with osteoarthritis, suggesting that iron was present and responsible for the effects. Histological analysis of cartilage confirmed iron deposition within chondrocytes, associated with low UTE-T2* values.

Conclusions: Iron accumulation can occur in cartilage (not only in synovium) and shows a clear association with joint health. Cartilage iron is a novel biomarker which, if quantifiable with innovative joint-specific MRI T2* sequences, may guide treatment optimization.

KEYWORDS

arthropathy, cartilage, haemophilia, hemophilia joint health score, hemosiderin, iron, magnetic resonance imaging

1 | INTRODUCTION

Arthropathy caused by frequent spontaneous joint bleeding is a progressive and debilitating co-morbidity in haemophilia.[1] The most salient features of haemophilic arthropathy are intra- and periarticular soft tissue inflammation with hypertrophy and osteochondral destruction.[2-5] In addition to insufficient plasma clotting factor activity levels, vascular instability caused by neovascularization, vessel remodelling and abnormal vessel leakiness may fuel a vicious cycle of re-bleeding, thereby promoting arthropathic progression.[3,6-8]

Supported by observations from explanted cartilage and haemophilia mouse and dog models, the concept has emerged that iron released from red cells and subsequently stored as hemosiderin in articular soft tissues creates a 'toxic' environment.[9] As a consequence, dysregulation of joint tissue metabolism, synovial inflammation and hypertrophy occur, ultimately resulting in cartilage destruction.[5] However, a more exact understanding of the influence of joint iron deposition on clinical outcomes and the progression of arthropathy in relation to imaging findings in patients with haemophilia (PWH) is lacking.

The purpose of this study was to evaluate joints of adult PWH using conventional and quantitative ultrashort time-to-echo (UTE) magnetic resonance imaging (MRI) sequences to delineate imaging pathology according to IPSG recommendations,[10] which also assess hemosiderin content semi-quantitatively, and to determine whether findings correlate with Hemophilia Joint Health Scores (HJHSs) and pain. Ultimately, we felt that such knowledge would improve our clinical understanding of haemophilic arthropathy and help to optimize the use of clinical and radiological assessments for therapeutic management.

2 | MATERIALS AND METHODS

2.1 | Patient population and data extracted

Adult patients with haemophilia A or haemophilia B of all severities (denoted as severe, moderate or mild depending on intrinsic factor FVIII or IX plasma activity of <1%, 1%-5% or ≥5%, respectively), age 21 years and older (n = 23), and seen consecutively during routine clinic visits over a 4-month period, underwent MRI examination of one painful or uncomfortable joint. At inclusion, Hemophilia Joint Health Scores (version 2.1) of the affected joints[11] (HJHS, 0 best, 20 worst) were determined for the affected joint by a licensed physical therapist with >5 years of general practice experience and approximately 2 years of experience with haemophilia patients. The physical therapist was trained in the HJHS acquisition according to instructions and guidance provided by online training and video modules developed by the International Prophylaxis Study Group (http://www.ipsg.ca/publication/hemophilia-joint-health-score-instructional-video-and-manual). Pain was self-assessed by visual analogue scale (VAS; 0 no pain; 10 worst pain). In terms of patient demographics, only age, type and severity of haemophilia were extracted from the electronic medical record. The study protocol, data acquisition and patient confidentiality safeguards were approved by the Human Research Protection Program (HRPP) at the University of California San Diego (UCSD), and patients provided written informed consent.

2.2 | MR imaging

MR imaging was performed on a clinical 3T scanner (Signa HDx, GE Healthcare Technologies) and either an 8-channel knee coil, 4-channel ankle coil or an 8-channel flexible surface coil (for knees, ankles and elbows, respectively) using the following 2D sequences: sagittal fast spin-echo (FSE) T1-weighted (650/10 ms; echo-train length of 4; 4 mm slice thickness; 0.5 mm interslice gap; 384 × 320 matrix; 14 cm field of view; and 1 signal average), sagittal FSE T2-weighted with fat suppression (4000/65 ms; echo-train length of 12; 4 mm slice thickness; 0.3 mm interslice gap; 384 × 288 matrix; 14 cm field of view; and 2 signal averages), coronal FSE T1-weighted (650/10 ms; echo-train length of 4; 4-mm slice thickness, 0.5-mm interslice gap, 384 × 320 matrix, 14-cm field of view, and 2 signal averages), coronal FSE T2-weighted with fat suppression (5000/65 ms, echo-train length of 16, 4-mm slice thickness, 0.5-mm interslice gap, 384 × 320 matrix, 14-cm field of view, and 1 signal average), axial FSE T1-weighted (650/10 ms; echo-train length of 4; 4-mm slice thickness; 0.5-mm interslice gap; 320 × 288 matrix; 14-cm field of view; and 1 signal average) and axial FSE intermediate-weighted with fat suppression (3200/40 ms; echo-train length of 9; 4-mm slice thickness; 0.5-mm interslice gap; 320 × 288 matrix; 14-cm field of view; and 1 signal average). In addition, sagittal three-dimensional (3D) ultrashort echo time (UTE) images were acquired with a cones readout trajectory at four different echo times (TR/TEs, 15 ms/0.03, 2.8-3, 5.6-6 and 8.4-9 ms; flip angle = 11°; 4 mm slice thickness; 256 × 256 matrix, 14-cm field of view, time ~6 minutes).[12] Intravenous contrast was administered for select cases when clinically indicated.

2.3 | Image interpretation and data analysis

The International Prophylaxis Study Group (IPSG) MRI score[10] was applied by a fellowship-trained, musculoskeletal radiologist with 6 years of experience and a fourth-year radiology resident. Both were blinded to each other's scores as well as clinical scores. The soft tissue domain, composed of effusion/hemarthrosis, synovial hypertrophy and hemosiderin, has a maximum score of 9. The osteochondral domain, composed of surface erosions, subchondral cysts and cartilage degradation, has a maximum score of 8. The IPSG MRI score has a maximum combined score of 17. The presence and extent of hemosiderin deposition were determined by evaluating the multi-echo UTE sequence, with special consideration to avoid misinterpreting as hemosiderin chemical shift artefacts of the second kind for voxels containing both fat and water on out-of-phase TEs.[13]

Using the sagittal multi-echo UTE images, a slice extending through the centre of each articulation was selected, and regions of interest (ROIs) were carefully placed on the midportion of the

patellar and talar cartilage (weight-bearing articulations) on the selected slice. Most elbows exhibited severe osteochondral wear with little or no cartilage left for quantitative MRI analysis and therefore were excluded from further analyses. ROIs were selected to minimize volume averaging artefacts. T2* values were calculated using a Levenberg-Marquardt fitting algorithm developed in-house in MATLAB (The Mathworks Inc).

2.4 | Human joint tissue and histology

The harvesting of human tissues at the time of total knee replacement surgery was approved by the UCSD HRPP. To assess cartilage changes and iron deposition by histopathology, bone pieces containing articular cartilage were sawed into approximately 2 × 1 cm pieces and fixed in formalin for >1 week. Pieces were rinsed and demineralized by daily changes of 10% formic acid in the presence of 0.2% potassium ferrocyanide to achieve en bloc Perls' reaction before loss of ferric ions in the acid demineralization solution. Decalcification included 5 days of formic acid, followed by 5 days of saline rinsing. Pieces of decalcified Perls'-reacted tissue were cryoprotected in 30% sucrose and cryosectioned at 8 µm before counterstaining with haematoxylin or periodic acid-Schiff stain. To limit staining to only light red nuclei with haematoxylin, modified Harris haematoxylin (SH30, Fisher Scientific) was mixed 1:2 with 0.5% aqueous acetic acid and applied for only 1 minute before differentiation in 70% ethanol with 1% HCl.

2.5 | Statistical analysis

Descriptive statistics were used to describe the patient cohort. Correlations between cartilage (T2*) MRI findings with pain, HJHS and MRI IPSG score were achieved by Spearman rank test. The consistency between the two radiologists was evaluated with the intraclass correlation coefficient.[13]

3 | RESULTS

3.1 | Patient and joint characteristics

The cohort characteristics are summarized in Table 1. Briefly, 23 patients (median age 42 years, standard deviation [SDE] 15 years) were recruited (seven ankles, nine knees, seven elbows) and imaged with MRI. There were 18 patients with haemophilia A and 5 with haemophilia B. Only four patients had mild or moderate haemophilia; the other 19 patients had severe haemophilia. MRI scores were very similar between the two readers and were averaged. There was a high degree of agreement: 0.95 and 0.98 for soft tissue total and osseous total, respectively.

Mean HJHS, VAS pain score and MRI IPSG score were 5.5 (SDE 3.4, range 0-11), 3.0 (SDE 2.4, range 0-7) and 10.3 (SDE 3.9, range 0-16). When the IPSG MRI score was divided into soft tissue and osteochondral domains, the mean scores were 3.2 (SDE 2.0, range 0-8) and 7.0 (SDE 2.4, range 0-8), respectively.

TABLE 1 Patient and joint characteristics

Characteristic	
Patient (n)	23
Haemophilia A	18
Haemophilia B	5
Severity (n)	
Mild/Moderate	4
Severe	19
Age (y; mean [SDE])	41.6 (15.2)
Joints (n)	
Knee	9
Elbow	7
Ankle	7
HJHS (mean [SDE])	5.5 (3.4)
Pain (VAS score; main [SDE])	3.4 (2.8)
MRI IPSG Score (mean [SDE])	10.3 (3.9)
Soft tissue domain	3.2 (1.9)
Osteochondral domain	Mode[a] = 8

Abbreviations: HJHS, Hemophilia Joint Health Score; IPSG, International Prophylaxis Study Group; MRI, magnetic resonance imaging; SDE, standard deviation; VAS, visual analogue scale.
[a]This variable cannot be expressed as mean with SDE because of its distribution with a mode of 8 and a range from 0 to 8.5 (17 subjects have the same value of 8).

3.2 | Relationships between IPSG MRI scores, pain and HJHS

There were no significant correlations between VAS pain scores and HJHSs with the MRI IPSG scores, either total or divided into soft tissue or osteochondral sub-scores. Charting of data correlations revealed that advanced MRI findings, represented as total IPSG score, or subdivided into the osteochondral and soft tissue domains, could be present at low HJHSs already, or even at a HJHS of zero. However, most abnormal MRI scores appeared above a HJHS of 3 (Figure 1).

3.3 | Quantification of iron in cartilage by MRI T2* relaxation and the relation to HJHS

Most elbows exhibited severe osteochondral wear with little or no cartilage left for quantitative MRI analysis. Therefore, iron quantification in cartilage was only performed on weight-bearing joints (knee and ankles, n = 16) and revealed a wide distribution of T2* values, with a mean T2* relaxation time of 9.2 ms (range 3.0-14.6 ms) (Figure 2A). Strong inverse correlations of T2* relaxation times with HJHSs (r_s = −0.81, P < 0.001) and IPSG MRI scores (r_s = −0.52, P = 0.037) were noted (Figure 2 B/C), suggesting that iron was present in cartilage and that iron loading was associated with a higher degree of clinical and imaging-based arthropathic joint changes. When subdivided into osteochondral and soft tissue domains, the

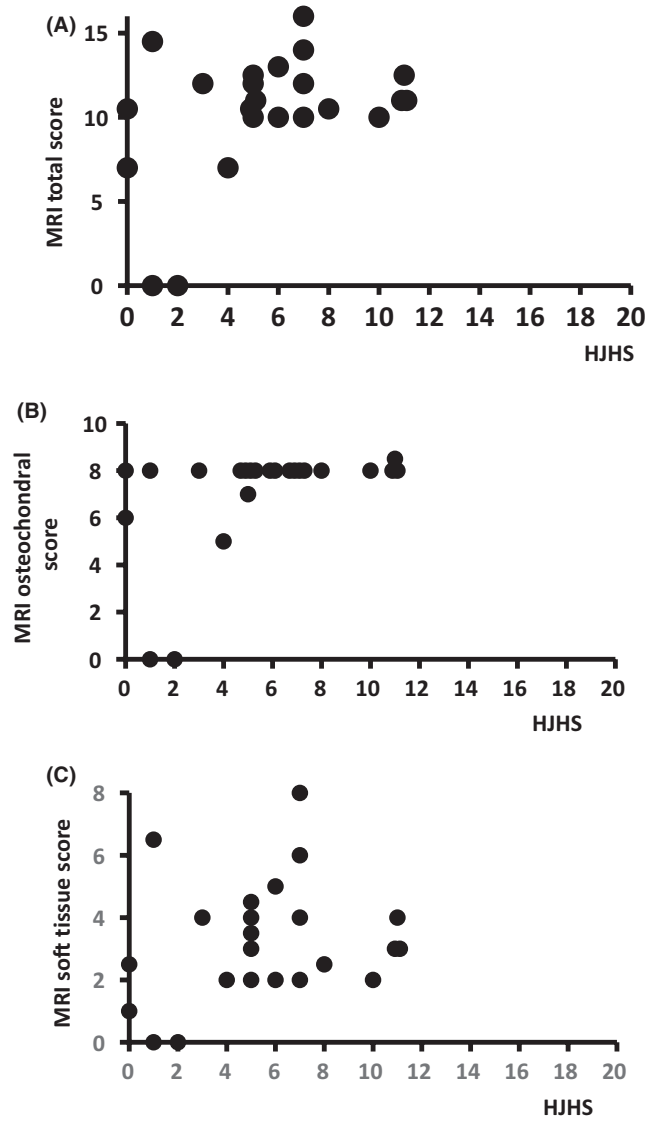

FIGURE 1 Relationship of HJHS and IPSG MRI scores. A, Total, B, osteochondral and C, soft tissue IPSG MRI scores were plotted against HJHS. HJHS, Hemophilia Joint Health Score; IPSG, International Prophylaxis Study Group; MRI, magnetic resonance imaging

MRI soft tissue score also correlated negatively (r_s = −0.46, P = 0.076) with T2* relaxation times. Correlation of the osteochondral MRI sub-score with T2* could not be performed on its own due to ceiling effects of the MRI score, whereby 10 of 16 MRIs yielded the highest score of 8. Two examples, depicting iron quantification in the knee joint of a patient with severe haemophilia A and another patient with mild haemophilia A, are shown in Figure 3.

3.4 | Histological analysis of explanted cartilage for iron loading

To prove iron accumulation in cartilage as suggested by MRI, we examined cartilage explants, harvested subsequently (within 2 years) from two of the patients undergoing total knee replacement surgery. The corresponding T2* relaxation times in the patellae were 4.1 and 4.7 ms, which were among the lowest measured, indicating high iron loading. Perls' reaction revealed substantial iron deposits within the cartilage, mostly within and around chondrocytes, but also in lacunae-like structures lacking any apparent active cell. Representative examples of one patient are provided in Figure 4.

4 | DISCUSSION AND CONCLUSION

Quantitative MRI has been widely implemented for non-invasive evaluation of hyaline articular cartilage.[14] Several studies have shown that chondral T2 and T2* relaxation times are sensitive to water content, collagen content and collagen fibril orientation.[15-17] Classically, elevated T2 values in cartilage have been considered to represent irreversible damage to the extracellular matrix.[18] Most recently, investigators have demonstrated that T2*, as measured by a multi-echo UTE technique similar to that used in this study, was significantly elevated in cartilage of injured knees compared with uninjured controls.[19,20] Based on these results from studies in non-haemophilic populations with injury or osteoarthritis, we expected to find a positive correlation between increasing T2* relaxation times and worsening joint status. However, we found a strong negative correlation between joint status determined by HJHS and MRI IPSG scores with relaxation times. This seemingly contradictory and surprising result may be explained by our histology results showing iron accumulation in the cartilage, which would decrease T2*.[21] The strong negative correlation suggests that the T2* shortening is a result of severe chondral iron deposition dominating the effects.

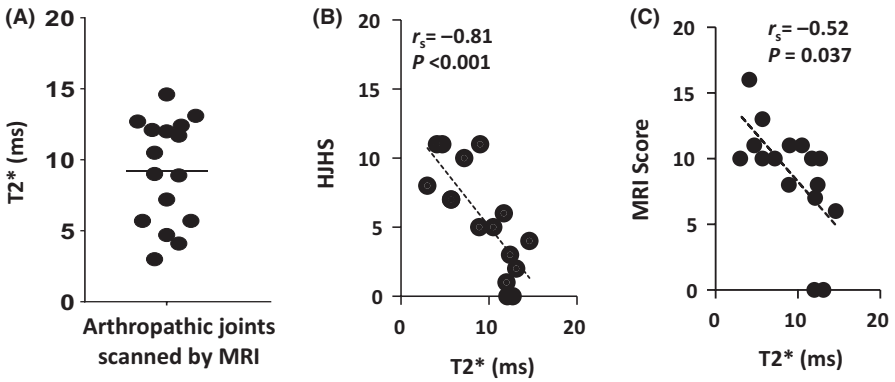

FIGURE 2 Quantification of iron in cartilage by MRI T2* relaxation in relation to HJHS and MRI scores. A, MRI T2* relaxation times were obtained from weight-bearing joints (knees and ankles, n = 16), and B, correlated with HJHSs and C, IPSG MRI scores by Spearman rank test. HJHS, Hemophilia Joint Health Score; IPSG, International Prophylaxis Study Group; MRI, magnetic resonance imaging; rs, Spearman rank correlation coefficient

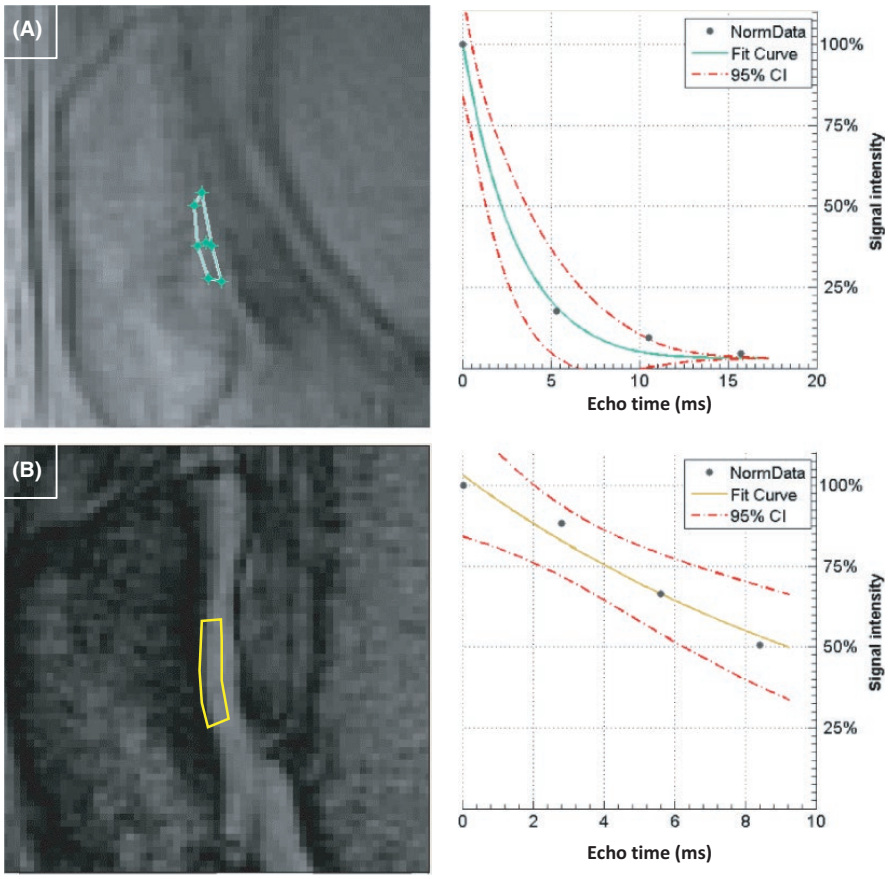

FIGURE 3 Magnetic resonance imaging quantification of iron in cartilage by T2*. Representative examples of A, high cartilage iron content (T2* = 3) in the knee of a patient with severe haemophilia A (HJHS 8; MRI IPSG score 10) and B, low cartilage iron content (T2* = 12.1) in the knee of a patient with mild haemophilia A (HJHS 1; MRI IPSG score 7). Left images demonstrate sagittal multi-echo UTE images with regions of interest drawn in the midportion of the patella. Right images demonstrate excellent curve fitting. HJHS, Hemophilia Joint Health Score; IPSG, International Prophylaxis Study Group; MRI, magnetic resonance imaging [Colour figure can be viewed at wileyonlinelibrary.com]

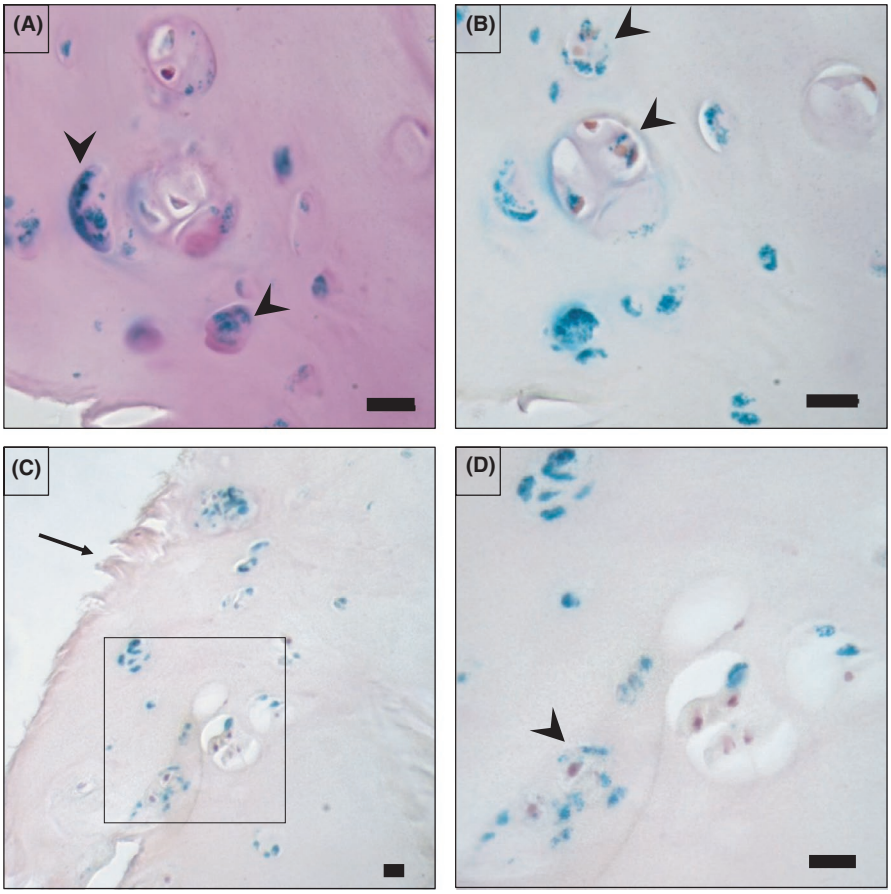

FIGURE 4 Histological depiction of iron accumulation in cartilage. Knee cartilage explants from a patient with haemophilia and osteoarthritis were examined histologically with Perls' reaction for iron content. A, Shows an iron-rich region counterstained with PAS, showing association of iron with chondron borders (25× objective). B, Same area counterstained for nuclei only with red haematoxylin on nearby section. C, Another area of wide iron distribution with red haematoxylin counterstain for nuclei (10× objective), with boxed region shown in D (25× objective). Fibrillation (arrow) and a paucity of chondrocytes are noted with many iron deposits (blue Perls' reaction product) in and around chondrocytes (example areas are denoted by arrow head). Bars 20 μm [Colour figure can be viewed at wileyonlinelibrary.com]

Iron deposition in chondrocytes of deteriorating cartilage in humans was described decades ago in several case series of patients with hemochromatosis.[22-24] However, not all studies analysing arthritic joints of patients with hemochromatosis have identified iron in cartilage while synovial iron was readily detectable.[23,25,26] It has been suggested that the paucity of observations of iron in cartilage may be due to the lack of sensitivity of osteochondral iron to usual iron stains (such as Prussian blue/Perls' stain) in histological examinations, since iron particles are small (0.25-0.5 μ) and may be beyond microscopic resolution, or to the loss of iron due to acidic fixation and decalcification techniques of joint tissue.[22] This has been elucidated by Ghadially et al[22] where iron detection with light microscopy after Prussian Blue/Perls' staining was compared to electron probe X-ray analysis of articular cartilage of rabbits after induced chronic hemarthrosis. In keeping with this observation, abundant synovial hemosiderin has been reported in haemophilic joints, whereas cartilage iron deposition has only been described in one case by light microscopy.[27,28]To our knowledge, there is only one additional case report, describing iron accumulation in cartilage in association with chronic hemarthrosis, but without an evident cause such as haemophilia.[29] Taken together, it appears that iron can accumulate in cartilage with or without hemarthrosis, but little to nothing is known about the mechanistic and molecular processes of cartilage iron loading, or its clinical consequences.

In hemochromatosis, it has been proposed that iron might contribute to the degeneration of cartilage, but a direct relationship between joint iron accumulation and degree of arthritis has not been established.[28,30,31]

To date, there is no knowledge about the extent of iron accumulation in cartilage of haemophilic joints in relation to joint status, function or dynamics of joint deterioration.

Somewhat surprisingly, observations from this study demonstrated that there was *no* correlation between clinical (HJHS) and imaging (MRI) scores, with highly abnormal imaging findings present over a wide spectrum of clinical scores. Similarly, widely divergent and mostly poor correlations between MRI IPSG scores and HJHS were reported previously,[32-34] especially in relation to the soft tissue compartments,[32] possibly dependent on joint type and articulations involved.[34] These findings suggest that, while depicting joint deterioration, imaging abnormalities may have limited bearing on clinical joint status, which may be driven at least in part by individual pain perception and functional mobility. In aggregate, these observations corroborate that the severity of imaging findings can be dissociated from clinical/functional joint status as has been observed in other arthritic conditions.[35] However, when imaging was focused on chemical iron quantification using T2* relaxation, significant correlations between iron content and clinical scores as well as imaging scores became evident. Reasons are unclear, but also indicate that both are affected proportionally by cartilage iron loading. These findings suggest that (a) iron accumulation in association with joint bleeding occurs in cartilage and not solely in synovial tissue as previously believed and (b) that iron, once deposited in cartilage, may play an important role for direct and continued cartilage toxicity, destruction and the progression of haemophilic arthropathy. This is of importance since osteochondral changes in haemophilia are believed to be mediated more indirectly by the pro-inflammatory milieu created by iron in synovial fluid and the synovium[5,36] rather than by direct deposition of iron in cartilage. In that sense, the pathways of iron uptake into cartilage and iron retention, potentially causing damage from 'inside-out' rather than from 'outside-in', as well as the potential for reversibility of cartilage iron loading are unknown.

These findings suggest a need not only to study molecular pathways of cartilage iron loading and retention, but also to develop and validate sensitive MRI iron quantification methodologies specific to cartilage or other joint tissues for more immediate clinical application. A number of novel quantitative MRI techniques are in development [37,38] which, if adjusted for cartilage imaging, may provide sensitive methods to precisely quantify cumulative joint iron loading over time. Quantitative iron imaging would revolutionize the ability to recognize the consequences of subclinical bleeding and/or suboptimal treatment strategies in haemophilia. At present, hemosiderin quantification is only possible in a semi-quantitative fashion,[10,32] which lacks sensitivity for dynamic, incremental measurements.

MRI T2* has been performed previously to quantify iron loading of solid organs (liver and heart) in iron overload disorders such as hemochromatosis, hemoglobinopathies or other conditions of transfusion iron overload,[39,40] but has not been validated yet for iron loading of joint tissues. While our results strongly suggest that T2* imaging can detect cartilage iron, sequences will have to be adjusted, improved and validated for joints. The development of such appropriate T2* joint imaging sequences may come timely since the management of haemophilia is currently undergoing a paradigm shift. Traditionally, the reduction of symptomatic joint bleeding, often expressed as annual bleeding rate (ABR), has been the most important outcome parameter for clotting factor therapies. However, ABR may be insufficient to guide management decisions for (a) emerging non-factor therapies, such as the recently FDA-approved FVIII-mimetic emicizumab (Hemlibra®, Genentech) improving coagulation profiles and (b) gene therapies on the horizon with anticipated constant elevation of plasma factor activity levels.[41] Since these new therapies mitigate or abrogate the fluctuations between plasma peak and trough clotting factor activity levels, correction of subclinical bleeding (rather than overt clinical bleeding) and appropriate iron clearance from the joint is important. It is in this arena that hemosiderin quantitation in joints could become a valuable assessment tool to measure treatment success. In that sense, interval detection of accumulation of hemosiderin during long-term treatment plans, employing non-factor or clotting factor strategies, would indicate that the prescribed therapy may not be adequate. Optimization of therapy may be achieved by discussing improved compliance, switching strategies, and/or adjustments with respect to dosing and/or frequency of drug administration.

In summary, it appears important to recognize that hemosiderin accumulation occurs not only in synovium, but also in cartilage, a fact that may be generally underappreciated in haemophilia care due to difficult histological detection by conventional iron staining

methods. Since findings from our study revealed that the amount of cartilage iron is associated with deteriorating clinical and imaging joint status, we hypothesize that cartilage iron plays a significant role in the progression of haemophilic arthropathy. Therefore, cartilage iron could be considered a biomarker of joint health that may become directly quantifiable with innovative joint-specific MRI T2* sequences, thus guiding the adjustment of therapeutic strategies to optimize joint health in patients with haemophilia.

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DISCLOSURES

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

AUTHOR CONTRIBUTIONS

AvD and EYC designed the study and shared responsibilities for study oversight, coordination, data analysis and manuscript writing. RFWB performed all statistical analyses. EYC and ZTB performed MRI scoring. HJ, YM and JD performed MRI data analysis. JHW performed the histology and histological analysis. All authors critically reviewed the manuscript and approved it in its final version.

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